Wisconsin Department of Agriculture, Trade and Consumer Protection

Wisconsin Pest Survey Report

PYTHIUM SPECIES ASSOCIATED WITH SOYBEAN SEEDLINGS

The Wisconsin Department of Agriculture, Trade and Consumer Protection Pest Survey and Plant Industry Bureau Laboratory conducted disease surveys on soybeans from 2011 until 2017. This report documents *Pythium* species detected in soybean seedling roots. Soybeans in the early vegetative stages are often affected by this fungus-like organism, in a group called water molds or oomycetes. Pythium is most aggressive on early vegetative stages of soybeans. It can cause seed rot, damping-off and non-lethal root infections which can reduce plant growth and yield. Soybean seedlings were also infected with several other closely related oomycetes: Phytophthora, Phytopythium and Pythiogeton.

The survey shows that Pythium was present in almost all soybean fields (table 1) and that a great variety of species were found in root samples (table 2). As of 2017, 16 different Pythium species have been identified. A summary of Phytophthora species identified during our survey can be found on this website under “Early Season Soybean Root Rot Survey”.

Table 1 Year	Survey Dates	Total Fields Surveyed	Fields with Pythium
2011	6-14 to 7-14	15	13 (87%)
2012	5-29 to 7-2	49	49 (100%)
2013	6-17 to 7-18	52	49 (94%)
2014	6-6 to 7-16	57	57 (100%)
2015	6-2 to 6-30	50	50 (100%)
2016	6-7 to 7-13	53	NA
2017	6-9 to 6-30	55	53 (96%)

Includes two corn fields in 2013 and four corn fields in 2014.

Soybean seedling roots with root rot symptoms.

Table 2. Pythium species present in soybean roots	Percent infested / total surveyed fields
P. acanthicum	0.4%
P. aphanidermatum	1.5%
P. arrhenomanes	6.7%
P. attrantheridium*	7.8%
P. conidiophorum	3.3%
P. heterothallicum	5.9%
P. inflatum*	3.7%
P. intermedium	0.4%
P. irregulare	4.8%
P. kunmingense	0.7%
P. recalcitrans*	9.3%
P. sulcatum	2.6%
P. sylvaticum	25.9%
P. torulosum	1.8%
P. ultimum	1.5%
P. violae	0.4%
P. spp.	18.8%

*: new species, ***bold italic***: pathogenic on soybean

Methods - DATCP plant pathologists collected soybean seedlings from up to 55 fields, when soybeans were in the vegetative to early reproductive stages. From each randomly chosen field, seedlings were carefully dug up, selecting symptomatic plants or plants from areas prone to

flooding or soil compaction. A combined sample consisting of 20 seedlings from each field was diagnosed by the Plant Industry Laboratory. Seedling roots were washed thoroughly before root tissue was tested for Pythium using gene-based methods. Amplification of DNA from the cytochrome oxidase gene region (Martin 2000, Villa 2006) and sequence analysis allowed for fast and accurate identification to species level without performing time-consuming culturing and morphological observations necessary for classic pathogen identification.

Results - A total of 278 root samples were tested from 2011 to 2017. Ninety-seven percent (270 of 278) of samples tested positive for the genus Pythium and 79% (219 of 278) of samples could be identified to Pythium species level. Eighteen percent (51 of 278) of samples could be identified to genus (Pythium spp.) but not to species level. Table 2 lists all 16 different Pythium species identified during our surveys of Wisconsin soybean fields. These findings agree with species reported in four recent studies. Research teams in Ohio (Broders et al 2007), North Dakota (Zitnick-Anderson et al. 2015) and Minnesota (Radmer et al. 2017) investigated Pythium diversity and their effect on soybean and corn. An extensive study of oomycetes associated with soybean seedlings in North America (Rojas et al. 2017) identified 51 Pythium species. Twelve of 16 Pythium species detected during our survey caused seed rot and/or seedling root rot in at least one of these studies. These findings expand the number of species that may have a negative effect on soybean seedling establishment and may ultimately have an impact on yield.

P. sylvaticum, *P. irregulare, P. intermedium* and *P. ultimum* isolates are well known and considered most detrimental to soybean seed and seedling roots. *P. sylvaticum* was the most frequently found species in our survey, present in 25.9% of Wisconsin fields. Less often found were *P. irregulare* in 4.8%, *P. intermedium* in 0.4%, *P. ultimum* in 1.5% of fields. *P. torulosum* (5.6%) and *P. conidiophorum* (3.3%) have more recently been associated with soybean seedling disease. It's worth noting that all these species are pathogenic on corn seed and seedlings as well.

P. recalcitrans (11.7%) and *P. attrantheridium* (9.9%), are newly described species and, as far as we know, had never been reported in Wisconsin soybean fields until our survey in 2012. *P. recalcitrans* was reported on carrots in Michigan in 2010 (5), grape vine roots in South Africa and beets in Spain in 2008 (6). A seed assay conducted by Minnesota investigators determined that *P. recalcitrans* is pathogenic on both soybean and corn.

Several Pythium found during our soybean survey are historically associated with other hosts: such as *P. arrhenomanes,* with corn, and *P. heterothallicum,* with wheat. Species like *P. aphanidermatum* are very destructive on greenhouse plants and infect a large variety of hosts (Farr and Rossman 2013, Van Der Plaats-Niterink 1981). *P. violae* causes cavity spot on carrots (Schrandt 1994). Pathogenicity studies showed that under warmer spring condition (68F) many species, including *P. aphanidermatum,* were more virulent than under cooler conditions (55F).

Two oomycetes were identified as *Pythiogeton (ramosum)* and *Phytopythium sp.* They belong to genera that are closely related to Pythium and Phytophthora (Von Minden 1916, Bala et al 2010) and share physical characteristics of both of these better-known water molds. *Pythiogeton ramosum* was blamed for soft rot symptoms on ginger roots. Pathogenicity was confirmed on carrot, potato, beans, pepper, cauliflower and sweet potato under high temperatures in Australia (Le et al 2014).

Identification of Pythium to species level has become more feasible for diagnostic laboratories since gene-based methods have become more readily available and affordable. Although our survey may not account for all species present, it documents a remarkable diversity of Pythium species in Wisconsin soybean fields.

Variability in species and multiple species infections can complicate effective control. In fields with a history of seedling disease an integrated management approach may be needed to control disease, including consideration of rotation strategies, given the reported host range of several of these species. For further information please see the University of Wisconsin Field Crops Pathology website, http://fyi.uwex.edu/fieldcroppathology/.

Literature:

Bala K, Robideau GP, Lévesque CA, et al. 2010. Phytopythium Abad, de Cock, Bala, Robideau, Lodhi & Lévesque, gen. nov. and Phytopythium sindhum Lodhi, Shahzad & Lévesque, sp. nov. Fungal Planet 49. Persoonia 24:136–137.

Broders, K. D., Lipps, P. E., Paul, P. A., Dorrance, A. E. 2007. Characterization of Pythium spp. associated with corn and soybean seed and seedling diseases in Ohio. Plant Disease 91:727-735.

Farr, D.F., Rossman, A.Y. 2013. Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA.

Le, D.P., Smith, M. & Aitken, E. First report of *Pythiogeton ramosum* (Pythiales) in Australia. Australasian Plant Dis. Notes (2014) 9: 130.

Lu, X.H., Jiang, H.H., Hao, J. J. 2013. First Report of Pythium recalcitrans causing cavity spot of carrot in Michigan. Plant Disease 97(7): 991.

Martin, F.N. 2000. Phylogenetic relationship among some Pythium species inferred from sequence analysis of the mitochondrially encoded cytochrome oxidase II gene. Mycologia 92 (4):711-727.

Moralejo E., Clemente, A., Descals, E., Belbahri, L., Calmin, G., Lefort, F., Spies, C. F. J., McLeod, A. 2008. Pythium recalcitrans sp. nov. revealed by multigene phylogenetic analysis. Mycologia 100 (2), pp. 310-319.

Rojas, J. Alejandro, Janette L. Jacobs, Stephanie Napieralski, Behirda Karaj, Carl A. Bradley, Thomas Chase, Paul D. Esker, Loren J. Giesler, Doug J. Jardine, Dean K. Malvick, Samuel G. Markell, Berlin D. Nelson, Alison E. Robertson, John C. Rupe, Damon L. Smith, Laura E. Sweets, Albert U. Tenuta, Kiersten A. Wise, and Martin I. Chilvers Oomycete Species Associated with Soybean Seedlings in North America—Part I: Identification and Pathogenicity Characterization. Phytopathology, March 2017, Volume 107, Number 3, Pages 280-292

Schrandt, J. K, Davis, R. M. and Nunez, J. J. 1994. Host range and influence of nutrition, temperature, and pH on growth of *Pythium violae* from carrot. Plant Disease 78, 335-338.

Van Der Plaats-Niterink, J. 1981. Studies in Mycology. No 21. Monograph of the Genus Pythium.

Villa N. O., Kageyama K., Asano K, Suga H., (2006) Phylogenetic relationships of Pythium and Phytophthora species based on ITS rDNA, cytochrome oxidase II and b-tubulin gene sequences. Mycologia, 98(3) pp. 410–422.

Von Minden, M.1916. Beiträge zur Biologie and Systematik einheimischer submerser Phycomyceten. Mykologische Untersuchungen und Berichte. 2:146-255.

Zitnick-Anderson, K. K., and Nelson, B. D., Jr. 2015. Identification and pathogenicity of Pythium on soybean in North Dakota. Plant Dis. 99:31-38.

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